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Buoyancy in Marine Fishes: Direct and Indirect Role of Lipids[1]

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SYNOPSIS. **The major lipids that have a direct role in buoyancy of marine fish are wax esters, squalene, and alkyldiacylglycerols. Wax esters are stored extracellularly in certain fishes, such as the orange roughy (*Hoplostethus atlanticus*), and therefore buoyancy appears to be their sole function. Some myctophid fishes have wax-invested swimbladders, where the non-compressible wax esters may aid in diurnal vertical migration, by replacing compressible swimbladder gases. Squalene is metabolically inert in the livers of certain sharks, and therefore probably has buoyancy as its only function. Alkyldiacylglycerols (DAGE) are abundant components of liver oil of certain deep sea sharks and holocephalans, where they may have an important role in buoyancy. Triacylglycerols and cholesterol are lipids that have an indirect role in buoyancy of marine fish. Many fishes in the ocean have oil-filled bones (mostly triacylglycerols). Although this oil aids buoyancy, its major function is as an energy storage lipid which can be utilized during starvation. Cholesterol, which is found in high amounts in the lipid-rich membranes of the swimbladder of deep sea fishes, may aid buoyancy by combining with oxygen gas in the swimbladder membranes to facilitate gas secretion in fish at great depths in the ocean. Further research is needed to understand the physical state of lipids, such as wax esters at deep sea temperatures and pressures, and more evidence is needed to clarify the role of cholesterol-rich membranes in swimbladders of deep sea fishes.**

INTRODUCTION

Strategies for buoyancy regulation in marine fishes are diverse. Positive buoyancy occurs in air-breathing fishes and swordfish when resting at the surface. Negative buoyancy is characteristic of benthic organisms which remain on the sea floor. Negatively buoyant benthic fishes, such as rays, skates, and flounders, must exert energy to swim off the bottom and remain afloat. Neutral buoyancy is often advantageous because organisms such as mid-water fishes and zooplankton can hover motionless in sea water and exert very little energy. To achieve neutral buoyancy, marine fishes have evolved numerous strategies including reduction of heavy ions and solutes of high partial molal volume which contribute to positive buoyancy in elasmobranchs. A gas-filled bladder helps fish and some invertebrates achieve neutral buoyancy. Maintenance of tissue water hypotonic to sea water, and storage of low density materials, such as lipid (0.86–0.93 g/ml), are also important in buoyancy.

The lipids most commonly used for buoyancy purposes by marine fish are squalene, wax esters, alkyldiacylglycerols, and triacylglycerols (Fig. 1). Squalene is metabolically inert in the livers of certain sharks (Nevenzel, 1989), and therefore probably has buoyancy as its only function. Because wax esters and alkyldiacylglycerols are used for metabolism at lower rates than triacylglycerols (Patton *et al.*, 1975), they may play a more important role in buoyancy than triacylglycerols. They have lower densities and greater positive buoyancy (Table 1). Wax esters have 0.165 g of positive buoyancy per ml lipid, alkyldiacylglycerols have 0.135 g per ml, and triacylglycerols have 0.095 g per ml (Table 1). When lipid is stored extracellularly, such as wax esters in orange roughy (Phleger and Grigor, 1990), it functions primarily in buoyancy because lipase activity only occurs within

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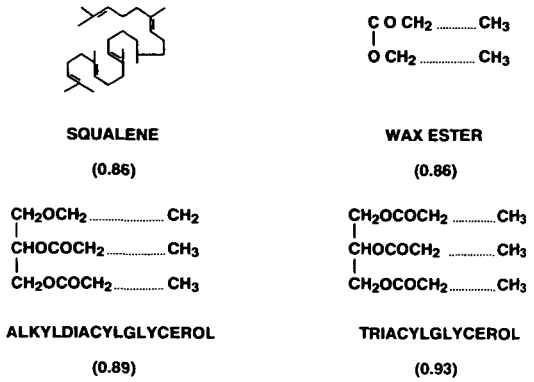

FIG. 1. Major lipids used for buoyancy in fishes. The specific gravities are (g/ml) given for wax esters, alkyldiacylglycerols, and triacylglycerols of similar chain length and unsaturation at 21°C (Sargent, 1989) and for squalene.

the cell. There are a number of reviews on buoyancy (Bone and Marshall, 1982; Phleger, 1991; and Alexander, 1993), and buoyancy is often discussed in reviews of lipid functions (Sargent, 1976, 1989; Lee and Patton, 1989).

The purpose of this paper is to discuss aspects of lipids and buoyancy in marine fishes. This is not a comprehensive review of lipid, nor is it a comprehensive review of buoyancy. Lipids that play a direct role in buoyancy, including wax esters, squalene and alkyldiacylglycerols, are discussed with appropriate examples. Lipids that play an indirect role are also discussed with respect to their function in swimbladders and oil-filled bones of marine fishes.

DIRECT ROLE OF LIPIDS IN BUOYANCY

Wax esters function in marine organisms as energy reserves and as buoyancy agents (Nevenzel, 1970). The function of wax esters is mostly as an energy reserve, with buoyancy as a secondary function (Lee and Patton, 1989). However, some fishes, such as the mid-water myctophids, appear to use wax esters primarily for buoyancy purposes.

Wax-invested swimbladders

The fat-investment of regressed swimbladders in certain mid-water and deep-water fishes involves accumulation of fat between the peritoneum and the tunica externa of the swimbladder. *Cyclothone atraria,* a mid-water gonostomatid fish, has a fat-invested swimbladder with 52% lipid (as percent dry weight) of which 78% is wax esters (Kayama and Ikeda, 1975). Lipids take on a major buoyancy function with age in myctophids as the swimbladder regresses and becomes invested with fat (Butler and Pearcy, 1972). Many of these fishes with regressed swimbladders also have massive deposits of wax esters in the body. The presence of high levels of wax esters (82–91%, as percent of lipid in whole fish) in myctophid fishes was first recognized by Nevenzel *et al.,* (1969) for *Triphoturus*

TABLE 1. *Densities of some common lipids and tissues of marine telosts at 1 atmosphere pressure and 20°C. Buoyancy of lipids in sea water (density 1.025 g/ml).*

Substance	Density (g/L)	g Buoyancy/ml lipid[c]
Lipid[a]		
Triacylglycerols	0.93	+0.095
Alkyldiacylglycerols	0.89	+0.135
Squalene	0.86	+0.165
Wax esters	0.86	+0.165
Cholesterol	1.07	−0.045
Tissue[b]		
Muscle	1.06	−0.035
Bone	1.30–1.50	−0.275, −0.475
Skin	1.05–1.07	−0.025, −0.045
Liver	0.99–1.05	+0.035, −0.025

[a] From Phleger (1991).
[b] From Alexander (1993).
[c] From Sargent (1976).

mexicanus, Lampanyctus ritteri, and *Stenobrachius leucopsarus* from the ocean off California and Hawaii. Wax esters comprised 86–91% of total lipid in the myctophid *Electrona antarctica* from the Antarctic Ocean (Phleger *et al.,* 1997). Similar high levels of wax esters occur in other myctophids including *S. nannochir* and *L. regalis* (Saito and Murata, 1996). Nine species of the 41 myctophid species analyzed for lipid by Neighbors (1988) have greater percentages of wax esters than triacylglycerols.

Adult myctophids make extensive diurnal vertical migrations to feed at night. Because the wax esters in a fat-invested swimbladder are incompressible, these fishes do not have to compensate for changes in pressure-induced wax ester volume during vertical migration. Wax esters are also less dense than triaclyglycerols (0.86 g/ml versus 0.93 g/ml) and their buoyancy in sea water (at a density of 1.025 g/ml) is higher, 0.165 g ml lipid versus 0.095 g ml lipid for triacylglycerols (Sargent, 1976). Although wax esters provide more static lift than triacylglycerols and are better for buoyancy, wax esters are hydrolyzed and reesterified four times slower than triacylglycerols (Patton *et al.,* 1975), and thus are not as efficient as a source of energy, depending on the metabolic rate.

The deep sea orange roughy, *Hoplostethus atlanticus,* also has a regressed swimbladder invested with wax esters (Phleger and Grigor, 1990) similar to the coelacanth, *Latimeria chalumnae* (Nevenzel *et al.,* 1966). The castor-oil fish, *Ruvettus pretiosus,* is another deep sea fish rich in wax esters (Nevenzel *et al.,* 1965). Wax esters are extracellular in fish such as orange roughy (Phleger and Grigor, 1990) and extracellular lipid may have buoyancy as its sole function because lipases are only intracellular (Phleger, 1991).

Wax esters are characterized by high levels of monounsaturated fatty acids (MUFA) (Sargent *et al.,* 1977). Saito and Murata (1996) reported 65.4–87.7% MUFA in myctophids, similar to levels observed in *Electrona antarctica* (Phleger *et al.,* 1997). MUFA comprised over 90% of fatty acids in the orange roughy (Bakes *et al.,* 1995; Elliot *et al.,* 1990). Fatty acids of the wax esters are low in polyunsaturated fatty acids. Fatty alcohols are generally dominated by the saturated palmitic acid (16:0) and myristic acid (14:0) with oleic acid (18:1n-9c and 18:1n-7c) being the major monounsaturated fatty alcohol.

Squalene in the liver of sharks

Squalene occurs in high quantities in the liver oil of various sharks, particularly those of the family Squalidae (Nevenzel, 1989). Huge livers characterize these sharks (20–30% of body mass) with more than 80% lipid (by weight) in the liver (Alexander, 1993). Squalene may comprise 40–90% of this liver oil, and comprises 15.5% of the body weight in *Centrophorus* spp. (Nevenzel, 1989). Squalene comprised 31.6–78.8% of the liver oils in seven species of deep sea sharks from Tasmanian waters (Deprez *et al.,* 1990). A high squalene content (50–82% of oil) was found in *Centrosymnus scalpratus, C. crepidator, Deania calcea,* and *Etmopterus granulosus* (Bakes and Nichols, 1995). One of these deep sea sharks (*E. granulosus*) contained high levels of both squalene and diacylglyceryl ethers. The eulachon, *Thaleichthyes pacificus,* is the only fish containing appreciable amounts of squalene (17% in the liver, Ackman *et al.,* 1968). Buoyancy is the only known function for squalene in shark livers, because it is essentially inert metabolically.

Squalene, an open chain isoprenoid hydrocarbon, is synthesized from acetate via mevalonic acid and is a direct precursor in the enzymatic synthesis of cholesterol. Squalene is usually found in only small amounts in the livers of higher animals where it is synthesized. In shark livers, which contain massive amounts of squalene, the formation of squalene-2,3 epoxide and lanosterol from squalene is inhibited, and squalene accumulates. Experiments involving starvation of sharks support the buoyancy hypothesis for squalene (Baldridge, 1970). Sharks lack a swimbladder and are typically negatively buoyant. The pectoral fins and heterocercal tail help keep them from sinking while swimming. The low density of squalene (0.86 g/ml) is about the same as wax esters.

Alkyldiacylglycerols

The alkyldiacylglycerols differ from triacylglycerols by having an alkyl linkage at carbon 1 of glycerol (Fig. 1). They are also termed glyceryl ethers or diacylglyceryl ethers (DAGE). The non-saponifiable neutral lipids from DAGE yield 1-0-alkylglycerols, also referred to as alkylglyceryl ether diols.

Alkyldiacylglycerols (DAGE) are abundant components of liver oil of certain shark species (Kayama *et al.,* 1971). The leafscale gulper shark (*Centrophorus squamosus*) has 79% DAGE in its liver oil (Deprez *et al.,* 1990). The Plunket shark (*Centroscymnus plunketi*) has 76.6% DAGE in the liver oil and the Pacific sleeper shark (*Somniosus pacificus*) has 49.5% DAGE (Bakes and Nichols, 1995). The liver of many of these sharks is over 50% oil; in some it is over 80% (Davenport and Deprez, 1989). The dogfish (*Squalus acanthias*) has 38–45% DAGE in the liver oil and other *Squalus* species may have up to 54% DAGE (Sargent, 1989). The holocephalans *Hydrolagus colliei, Chimaera barbouri,* and *C. ogilbye* have more than 50% DAGE in their liver oil (Sargent, 1989) and *H. novaezealandiae* has 66% DAGE in its liver oil (Hayashi and Takagi, 1980). Methoxy-glyceryl ethers, first isolated from the lipid of the liver of Greenland shark, account for 0.1–0.3% of liver lipid in some sharks (*S. acanthias, Daliatias licha,* and *Scyliorhinus torazame*) and ratfish (*H. novaezealandiae, H. barbouri,* and *Rhinochimaera pacifica*) (Hayashi and Takagi, 1982). These compounds have antibiotic activity and inhibit the growth of tumors in mice (Hayashi and Takagi, 1982).

A convincing argument for buoyancy as a function of DAGE was made by Malins and Barone (1970), who found that concentration ratios of DAGE to triaclyglycerols to increased in the liver of weighted *Squalus acanthias* (from weighted whole fish) when compared to an unweighted control group (weighted fish had 114 g lead weights suspended between the pectoral fins). One g of DAGE gives 14% more buoyancy than one g of triacylglycerols (Malins and Barone, 1970). *S. acanthias* may regulate buoyancy by selective metabolism of DAGE and triacylglycerols during vertical migrations. More experimental data are needed to prove conclusively that DAGE is used to regulate buoyancy (Sargent, 1989).

INDIRECT ROLE OF LIPIDS IN BUOYANCY

Cholesterol has a density (1.067 g/ml) greater than sea water (1.025 g/ml). Nevertheless, cholesterol is a major lipid in swimbladders of deep sea fishes and therefore has an indirect role in buoyancy. Oil-filled bones of fishes usually have triacylglycerols as the major lipid class. Because the principal role of triacylglycerols is energy storage, they would have an indirect role in buoyancy.

Swimbladder

The gas-filled swimbladder of osteichthyean fish functions primarily as a buoyancy organ (Pelster and Scheid, 1992; Alexander, 1993; Phleger, 1991). Fishes with a physostomous swimbladder, which has a connection via the pneumatic duct to the gut, may fill their swimbladders by swallowing air at the surface and "burp" it out. A physoclistous swimbladder has no connection to the gut, and gas secretion and absorption must occur within the swimbladder itself. The discussion here will concentrate on gas secretion in swimbladders and the function of lipid in physoclistous swimbladders.

Gas secretion in swimbladders.—Physostomous fishes such as the clupeoids may obtain gas by gulping air into the gut at the surface. The air is then passed to the swimbladder from the gut via a pneumatic duct. In the beluga sturgeon, *Huso huso,* the first filling of the swimbladder probably occurs by intestinal gases released during food digestion (Tsvetkov and Sbikin, 1983). When oceanic herring (*Clupea harengus*) descend to depths of 100–250 m, swimbladder gas is conserved by guanine crystals in the swimbladder wall which decreases gas diffusion (Blaxter and Batty, 1984).

The problem of gas secretion by fish into their swimbladders at great depths in the ocean is still not fully understood. Half of the benthic fishes found at depths greater

than 2,000 m have functional swimbladders. In fishes living below 40–50 m depth in the ocean, oxygen often comprises 90% or more of the swimbladder gases, with the remainder including nitrogen, argon, and carbon dioxide. A functional swimbladder in a brotulid fish at 7,000 m (Nielsen and Munk, 1964) would require 630 atm oxygen tension (90% of the total). This fish is therefore capable of increasing the oxygen tension from less than 0.8 atm (in sea water at great depths; Enns *et al.,* 1965) to 630 atm in its swimbladder. This remarkable feat has never been satisfactorily explained.

The secretion of oxygen occurs by release from blood oxyhemoglobin, caused by the Root effect. The Root effect involves a decrease in the oxygen carrying capacity in the blood of up to 50%, due to decreasing pH caused by lactic acid and CO_2 which are formed from glucose in the swimbladder gas secreting gland (Pelster and Scheid, 1992). The anaerobic glycolytic production of lactic acid and CO_2 occurs under hyperbaric oxygen conditions in the gas gland cells of the swimbladder (D'Aoust, 1970), which are somehow insensitive to high partial pressures of oxygen. CO_2 production also lowers pH and stimulates the Root effect. Both lactate and bicarbonate cause a salting-out effect of blood gases into the swimbladder. However, this salting-out effect only allows for a reduction in gas solubility in the bladder vessels of 1% or less (Pelster and Scheid, 1992; Gerth and Hemmingsen, 1982).

Primary concentration of oxygen gas by the Bohr or Root effect and salting-out is maintained and multiplied by countercurrent exchange in the rete mirabile, a tightly associated bundle of venous and arterial capillaries which circulate blood through the swimbladder gas gland. Retia are up to 25 mm in length in deep sea fish, compared to 5 mm or less in physoclist fishes from shallow water. Despite effective countercurrent multiplication, the Root effect for oxygen secretion in deep sea fishes is limited by back diffusion due to oxygen and no longer has an effective role in oxygen unloading to the swimbladder at pressures in excess of 40 atm in the swimbladder (Enns *et al.,* 1967). Consequently, Noble *et al.* (1975) were unable to explain the filling of the swimbladder in the flatnose codling, *Antimora rostrata,* at one mile depth.

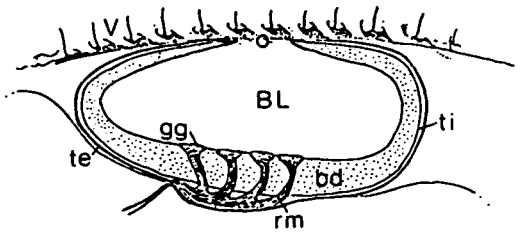

FIG. 2. The swimbladder of *Coryphaenoides armatus,* longitudinal section, illustrating the fatty bladder deposit (bd) as it appears in deep sea fishes which have not experienced rapid decompression (from Bowne, 1982). V = vertebra, BL = swimbladder lumen, te = tunica externa, gg = gas gland, rm = rete mirabile, o = oval, ti = tunica interna.

Fat-filled swimbladders.—Most deep benthopelagic fishes that live at depths in excess of 800 m in the ocean have fat-containing swimbladders that are still fully functional and contain hyperbaric oxygen gas (Phleger and Benson, 1971; Bowne, 1982). This fatty deposit appears in freshly captured fishes as a "foam" filled with bubbles of oxygen gas, and is often found partially ejected from the swimbladder by expanding gas from rapid ascent on long lines (Patton and Thomas, 1971). In macrourid fishes brought to the surface on slowly rising trawls, the swimbladder may not be ruptured by rapid decompression, and the fatty material is observed as a thick coat lining the inner surface of the swimbladder (Wittenberg *et al.,* 1980; Bowne, 1982; Fig. 2).

The lipid to protein ratio of this swimbladder foam is high at 1.5–2:1, and the lipid is rich in cholesterol and phospholipids (1:1 ratio; Phleger, 1991). The density of cholesterol is greater than seawater (Table 1). Fatty acids of the phospholipids are highly unsaturated (72–89%). The unsaturated fatty acids from swimbladder foam of five deep sea fish species (*Dicrolene intronigra, Bassozetus* sp., *Barathrodemus iris, Synaphobranchus brevidorsalis,* and *Aldrovandia gracilis*) included mostly oleic acid (18:1, 9–24%), palmitoleic acid (16:1, 12–23%), C-20, C-22 unsaturated fatty acids (13–32%), and (24:1, 6–13%) (Phleger

et al., 1978; 18:1 means 18 carbon atoms with one double bond, and unsaturated means one or more double bonds). These unsaturated fatty acids resist auto-oxidative processes in the swimbladder which may contain hundreds of atmospheres pressure of oxygen gas. Superoxide dismutase is the only enzyme capable of degrading oxygen radicals found in elevated amounts in swimbladder gas glands of fish (Morris and Albright, 1984). However, the organization of the lipids into gram quantities of pure bilayer membrane (membranes are the only structures visible by electron microscopy in the swimbladder foam) may also help confer protection against auto-oxidation (Phleger, 1991). Mitochondria, which are very susceptible to oxygen damage, are absent from the swimbladder foam which appears to be extracellular. The pentose-phosphate shunt (intracellular) may provide protection against auto-oxidation, because it results in formation of NADPH. Glutathione reductase, an intracellular radical-oxidizing enzyme that uses NADPH as a coenzyme, has been measured in eel swimbladders at levels far in excess of those in eel muscle (Pelster and Scheid, 1992). A transparent, semi-solid gelatinous tissue has also been observed to line the walls of the swimbladder of morid (*Physiculus* sp.) and ophidiiform fishes (*Brotula barbatum* and *Bassozetus* sp.). The deposit may be analogous to the lipid foam because it has an intimate relationship with the gas gland (Bowne, 1982). A similar tissue has been observed in the swimbladder of the shallow water brotulids *Ophidion barbatum* and *O. rochei* (Svetovidov, 1961). A globular mass of submucosal tissue has been observed in the posterior-ventral floor of the swimbladder of the hoki, *Macruronus novaezelandiae* (Jay, 1993). This submucosal tissue was reported not to contain lipid (Jay, 1993).

Two hypotheses have been proposed for the function of this cholesterol-rich lipid membrane system in the swimbladders of deep sea fishes. The first hypothesis is that the lipid-rich swimbladder wall is a barrier to oxygen diffusion (Wittenberg *et al.*, 1980), and has the same function as guanine crystals in silvery layers of some other swimbladders. The swimbladder wall is brilliantly white and reflective in freshly captured specimens, suggesting that the lipids are highly ordered (Wittenberg *et al.*, 1980). The argument is that the swimbladder lipids are below their phase transition temperatures, and thus diffusion coefficients are one thousand-fold less than in fluid membranes (Wittenberg *et al.*, 1980). Addition of cholesterol to a phospholipid membrane also reduces the diffusion constant by an order of magnitude.

The second hypothesis proposes that oxygen dissolution in the swimbladder lipid reduces back diffusion due to oxygen on the gas gland, thus facilitating oxygen secretion by the Root effect (Phleger, 1991). Because the Root effect does not functionally transport oxygen at pressures in excess of 40 atm, Scholander (1954) had suggested that some substance must function in dissociation of oxygen from oxyhemoglobin at high pressures in the deep sea. Because the fatty acids of the swimbladder membranes are highly unsaturated, they are unlikely to be solid. Because they are not solid, they could not be an effective diffusion barrier. Lower melting points of unsaturated fatty acids help keep lipids at a liquid state at the lower temperatures (1–4°C) that deep sea fish experience. To clarify the functions of lipid in swimbladders, the oxygen solubility must be measured in swimbladder membranes at deep sea temperatures and pressures. Also, thermal transitions of the lipids of the swimbladder membrane must be determined directly. We do not even know if the oxygen is present as a gas phase in the swimbladder of the fish at the depth where it lives. To answer this question, it might be possible to dissect the fish swimbladder at depth by remote control and photograph the swimbladder contents.

Oil-filled bones

Because skeletal tissue may have a density of 1.3–1.5 g/ml (Table 1), some marine fish reduce bone density to attain neutral buoyancy. Deep sea fishes are characterized by a reduction in bone ossification, as well as protein reduction. Density regulation in sharks is aided by cartilage, which is secondarily derived from osseous endoskeletal tissue in ancestral forms. The lumpsucker

(*Cyclopterus lumpus*), a teleost fish, also has a cartilaginous skeleton, which has an average density of 1.04 g/ml (Davenport and Kjorsvik, 1986). Cartilage is also substituted for bone in the Antarctic toothfish (*Dissostichus mawsoni* which is neutrally buoyant (Eastman, 1979). Other Antarctic notothenioids, namely *Pleurogramma antarcticum, Pagothenia borchgrevinki,* and seven species of *Trematomous,* are characterized by skeletal reduction as an aid to the attainment of neutral buoyancy (Eastman and De Vries, 1982).

Oil-filled bones are common in fish. Fifty-five species of fish from 37 families have been examined to date, and 14 of these families have species with over 24% lipid in the skeleton (as percent weight) (Phleger and Wambeke, 1994). The bone lipid in the majority of these species is triacylglycerol. Various functions have been proposed for fish bone lipids. These include buoyancy, and in some cases the posture of the fish (the hawkfishes, *Cirrhitus* spp. and the castor oil fish, *Ruvettus pretiosus*; Phleger, 1987; Bone, 1972). Bone lipid is also used for energy during starvation.

The impressive migration and spawning of Pacific salmon into streams and rivers of the Pacific Northwest, British Columbia, and Japan involve total fasting with associated biochemical and morphological changes. Almost all body lipid is depleted in tissues such as flesh (Shimuzu and Kaeriyama, 1991) and bone (Phleger *et al.,* 1995). Polyunsaturated fatty acids (PUFA) of the skeletal triacylglycerols of the pink salmon, *Oncorhynchus gorbusha,* are selectively depleted during the spawning migration. Ocean prespawning salmon had 24% PUFA (primarily eicosapentaenioc acid and docosahexaenoic acid) whereas postspawning pink salmon contained only 9% PUFA (Phleger *et al.,* 1995). Associated with this selective PUFA depletion is a relative increase of 20 and 22 monounsaturated fatty acids (MUFA), which has also been reported in liver and viscera of chum salmon (Sasaki *et al.,* 1989). This selectivity of fatty acid mobilization during fasting appears to be a general phenomenon among vertebrates (Raclot and Groscolas, 1993; Groscolas, 1990). The mechanism of this selective mobilization of fatty acids from fat cells is not understood. Hydrolytic lipases may discriminate against monounsaturated fatty acids with 20 and 22 carbons during fatty acid mobilization, and these MUFA may then be stored in the bones as an energy reserve. Henderson and Tocher (1987) reported that B-oxidation in mitachondria of trout, as in mammals, discriminates against derivatives of the longer chain fatty acids. Thus, bone triacylglycerols are used as an energy source during starvation in salmon, and buoyancy is secondary in importance.

Skeletal lipid in the blackbelly rosefish (*Helicolenus dactylopterus lahillei*) is stored inside adipocyte cells (Mendez *et al.,* 1993). The poorly calcified bones of this fish had 13–28% lipid (as percent weight of the bone), and were oily and spongy to touch. Triacylglycerols comprised 84.4% of the oil content in the skull and 87.8% in the spine. Although wax esters were 21.1% of the muscle oil content, they were not detected in the spine nor the skull (Mendez *et al.,* 1993). The flesh wax esters and the poorly calcified bones probably were important in buoyancy control whereas the skeletal triacylglycerols contributed more to energy reserve.

The neutrally buoyant fish *Dissostichus eleganoides* has a skeleton with low mineralization in the vertebrae, which have cavities filled with lipid (Ozarzun, 1988). High lipid contents in *D. eleganoides* were also reported in adipose cells in subcutaneous and muscular deposits (Eastman, 1988).

Some fish store wax esters in their bones. The bones of the orange roughy are filled with wax (Grigor *et al.* 1983; Phleger and Grigor, 1990), and the bones of the coelancanth, *Latimeria chalumnae* and the castor-oil fish, *Ruvettus pretiosus* are probably also wax filled (Nevenzel *et al.,* 1966, 1965). The myctophid fish *Electrona antarctica* has oily bones (20.3–32.6% in the neurocranium) which contain primarily wax esters (67–88%) (Phleger *et al.,* 1997). The wax esters in these fishes reflects the body lipid composition of the fish. It is unusual to find bones containing wax esters; most fish with oily bones have triacylglycerols as

the major lipid class in the skeleton (Phleger and Wambeke, 1994).

CONCLUSIONS

Our understanding of the physiological roles of wax esters in deep sea fishes such as myctophids and orange roughy will be incomplete until we have an accurate understanding of the physical state of the wax esters at great depths. Temperature and pressure both have important effects on the state of lipid, percent solid and liquid. The phase of wax esters affects their density; solid wax is more dense than liquid wax.

Further research is needed on the distribution and function of diacylglyceryl ethers (DAGE) in marine organisms. More experimental evidence is needed to support a buoyancy role for DAGE in shark livers. Because DAGE is not metabolically inert like squalene in shark liver, a role in buoyancy is difficult to prove conclusively.

Some lipids, such as triacylglycerols in fish bones are used more as an energy reserve than for buoyancy. Fatty acids are used selectively during starvation in fish, birds, and mammals. The mechanism of this selective mobilization of fatty acids from fat cells is not understood. More research is needed to understand this process which may be related to selective hydrolysis of triacylglycerols in fat cells.

Despite much effort, oxygen secretion into fish swimbladders at great depths in the ocean has still never been fully explained. The fact that marine fish with functional swimbladders living below 800 m depth have a cholesterol-rich lipid deposit in their swimbladders with hyperbaric oxygen may be important. One hypothesis for this lipid deposit is that it provides a barrier to oxygen diffusion out of the swimbladder. This theory requires that lipids are below their phase transition temperature and have very low oxygen solubility. A second hypothesis suggests that the swimbladder lipids instead dissolve oxygen gas, thus reducing oxygen back pressure on the gas gland and facilitating the Root effect at great pressures in the deep sea. The dissolved oxygen contributes to buoyancy in the swimbladder. The fatty acids of the phospholipids in the swimbladder membrane are highly unsaturated, meaning that they must be above their phase transition temperature and, therefore, not provide a barrier to oxygen diffusion as proposed by the other hypothesis. To solve this question, we need measurements of the solubility of oxygen in the lipid membranes of the swimbladder at deep sea temperatures and pressures. Phase transition temperatures of these lipids must also be measured at ambient deep sea conditions. Finally, we need to determine whether a gas phase exists in swimbladders of deep ocean fish.

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